ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-CoV-2 ASSAY
(Biocollections Worldwide, Inc.)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 Assay will be performed at Biocollections Worldwide Inc., laboratory located at 5735 NE 2nd Avenue, Miami, Florida 33137-2507 which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per the Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to BioCollections Worldwide, Inc., certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test performed on the Abbott m2000 system. The test uses primers and probes targeting the nucleocapsid (N) gene of the SARS-CoV-2; the oligonucleotide sequences are identical to the primers and probes sequences of the CDC EUA assay. One primer/probe set is for the universal detection of SARS-like coronaviruses and the two other primer/probe sets are for the specific detection of the SARS-CoV-2.

RNA is isolated/purified from upper respiratory specimens and bronchoalveolar lavage (BAL) using the Abbott m2000sp instrument. The bound RNA is eluted and transferred to a 96 deep-well plate for amplification. The master mix is prepared manually and is added to a PCR plate together with the extracted RNA. The RNA is reverse transcribed to cDNA and then amplified in the Abbott m2000rt instrument. During the amplification steps, amplification products are denatured to single strands at high temperature allowing primer annealing and extension at lower temperatures. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye (FAM) to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity.

The primer and probe sequences for the SARS-CoV-2 Assay are listed in the table below:

Name	Description	Oligonucleotide Sequence (5'-3')	Label	Working solution
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	10 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	10 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	10 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	10 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	10 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	10 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNase P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	10 µM
RP-R	RNase P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	10 µM
RP-P	RNase P	5'-FAM – TTC TGA CCT GAA	FAM,	5 µM

	Probe	GGC TCT GCG CG – BHQ-1-3’	BHQ-1	

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 Assay is to be used with the Abbott automated m2000 System which combines the automated sample preparation system, m2000sp (software version 8.1.8.0) and the real-time PCR thermal cycler/reader system, m2000rt (software version 8.1.9.0).

REAGENTS AND MATERIALS

Reagent	**Manufacturer**	**Catalog #**
SuperScript III RT/Platinum Taq Mix	Invitrogen	11732-020
2019-nCoV_N1-F	Eurofins	Custom
2019-nCoV_N1-R	Eurofins	Custom
2019-nCoV_N1 probe	Eurofins	Custom
2019-nCoV_N2-F	Eurofins	Custom
2019-nCoV_N2-R	Eurofins	Custom
2019-nCoV_N2 probe	Eurofins	Custom
2019-nCoV_N3-F	Eurofins	Custom
2019-nCoV_N3-R	Eurofins	Custom
2019-nCoV_N3 probe	Eurofins	Custom
RP-F	Eurofins	Custom
RP-R	Eurofins	Custom
RP Probe	Eurofins	Custom
2019-nCoV-N Positive Control	IDT	10006625

CONTROLS TO BE USED WITH THE SARS-CoV-2 MOLECULAR DETECTION ASSAY

1) **Negative Control (NTC):** Viral transport media serves as a negative control (reagent contamination and extraction control) and is included in each run. This control contains all of the reagents for amplification but does not contain the targeted nucleic acid template.

2) **Positive Control (PTC):** The positive control is the 2019-nCoV-N Positive Control (IDT) that contains target sequences of SARS-CoV-2. It is an extraction/amplification control and is include in every run to ensure the reagents are functioning as expected.

3) **Human Specimen Control (HSC):** A known negative sample is used as HSC in each run. HSC is extracted concurrently with the test specimens, provides a nucleic acid extraction procedural control, and a secondary negative control that validates the nucleic extraction procedure and reagent integrity.

4) **RNase P Internal Control (IC):** RNase P in a clinical sample is considered as an internal positive control for extraction and amplification. All clinical samples and HSC should yield a positive result with the RNase P primers and probe set.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

1) ***SARS-CoV-2 Molecular Detection Assay Controls – Positive, Negative and Internal***

The expected results for each control with all the primer/probe sets are shown in the table below:

	Primers/Probe sets expected results			
Control	**2019-nCoV_N1**	**2019-nCoV_N2**	**2019-nCoV_N3**	**RP**
PTC	Positive (<40 Ct[1])	Positive (<40 Ct)	Positive (<40 Ct)	N/A
NTC	Negative (ND[2])	Negative (ND)	Negative (ND)	Negative (ND)
HSC	Negative (ND)	Negative (ND)	Negative (ND)	Positive (<40 Ct)

[1] Cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold.
[2] not detected

2) ***Examination and Interpretation of Patient Specimen Results:***

Assessment of clinical specimen test results should be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The table below lists the expected results for the SARS-CoV-2 Assay.

SARS-CoV-2 N1	**SARS-CoV-2 N2**	**SARS-CoV-2 N3**	**RNase P**	**Result Interpretation**	**Report**	**Actions**
+	+	+	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate public health authorities.
If only one or both targets are positive		+/-	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate health authorities.
-	-	+	+/-	SARS-CoV-2 is Inconclusive	INCONCLUSIVE	Sample is repeated once. If the repeated result remains "INCONCLUSIVE", additional confirmatory testing may be conducted if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
-	-	-	+	SARS-CoV-2 Not Detected	NEGATIVE	Report results to sender.
-	-	-	-	Invalid Result	INVALID	Sample is repeated once. If

						a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.

LIMITATIONS

- The use of SARS-CoV-2 Assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems may generate erroneous results.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decision. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- If the virus mutates in the real time RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- The performance of this test has not been established for monitoring treatment of COVID-19.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- A false positive result may arise from cross contamination during specimen handling or preparation, or between patient samples.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

PERFORMANCE EVALUATION

1) Analytical Sensitivity/Limit of Detection (LoD):

The LoD study established the lowest concentration of SARS-CoV-2 that can be detected by the SARS-CoV-2 Assay at least 95% of the time. A preliminary LoD for each primer and probe set (for three N gene targets) was determined testing triplicate spiked samples processed using Abbott m2000sp. The spiked samples were prepared by

spiking 10-fold serial dilutions of RNA transcripts purchased from Biosynthesis into pooled Nasopharyngeal swabs/Oropharyngeal swabs clinical matrix. The LoD was confirmed by testing 20 replicates for the three SARS-CoV-2 markers (N1, N2, and N3). The results are shown in the table below.

Targets	**2019-NCoV_N1**	**2019-NCoV_N2**	**2019-NCoV_N3**
RNA Conc (copies/µL)	1	1	1
Pos/Total	20/20	20/20	20/20
Mean Ct	33.7	36.8	34.9

The study results showed that the LoD of the SARS-CoV-2 Assay is 1 copy/µL.

2) Inclusivity/Analytical Specificity:

The sequences for the N1, N2, and N3 primers/probes used in this assay are identical to the N1, N2, and N3 primer/probe sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel.

3) Clinical Evaluation:

The clinical evaluation was performed by testing patient samples in a blinded fashion. The performance of SARS-CoV-2 Assay was established using 70 clinical specimens: 13 oropharyngeal (OP) swabs and 57 nasopharyngeal (NP) swabs. The positive and negative percent agreements were analyzed by comparing the SARS-CoV-2 Assay results to the FDA-authorized EUA test as shown in the table below. The results of the SARS-CoV-2 Assay showed 100% concordance with the FDA-authorized EUA test results. This testing fulfills the requirement for confirmatory testing of at least five positive and five negative specimens.

Clinical Performance of the SARS-CoV-2 Assay with Upper Respiratory Swab Samples (NP and OP swabs)

			FDA EUA Assay	
			Pos	**Neg**
SARS-CoV-2 Assay	**NP**	**Pos**	32	0
	OP		3	0
	NP	**Neg**	0	25
	OP		0	10
Positive Percent Agreement	100% (95% CI: 90.11% - 100%)			
Negative Percent Agreement	100% (95% CI: 90.11% - 100%)			

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the Abbott mSample Preparation System reagents and Abbott automated m2000 System. The results are summarized in the following Table.

Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	**Specimen Type**	**Product LoD**	**Cross-Reactivity**
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not Detected

WARNINGS:

• This test has not been FDA cleared or approved;
• This test has been authorized by FDA under an EUA for use by authorized laboratories;
• This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
• This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner